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AD-A224 534

DOCUMENTATION PAGE

Form Approved OMB No 0704-0188

(U)

ELECTE JUL 24 1990 S D

1b RESTRICTIVE MARKINGS: NA

2a SECURITY CLASSIFICATION AUTHORITY: NA

3 DISTRIBUTION/AVAILABILITY OF REPORT: Distribution Unlimited

2b DECLASSIFICATION/DOWNGRADING SCHEDULE: NA

4. PERFORMING ORGANIZATION REPORT NUMBER(S): University of South Alabama

5 MONITORING ORGANIZATION REPORT NUMBER(S): NA

6a NAME OF PERFORMING ORGANIZATION	6b OFFICE SYMBOL (If applicable)	7a NAME OF MONITORING ORGANIZATION
University of South Alabama	NA	Office of Naval Research

6c. ADDRESS (City, State, and ZIP Code)	7b ADDRESS (City, State, and ZIP Code)
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8a. NAME OF FUNDING/SPONSORING ORGANIZATION	8b OFFICE SYMBOL (If applicable)	9 PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER
Office of Naval Research	ONR	N00014-88-K-0429

8c. ADDRESS (City, State, and ZIP Code)	10 SOURCE OF FUNDING NUMBERS			
	PROGRAM ELEMENT NO	PROJECT NO	TASK NO	WORK UNIT ACCESSION NO
800 N. Quincy Street Arlington, VA 22217-5000	61153N	RR04108	441q801	

11 TITLE (Include Security Classification)
(U) Effects of Poloxamer 188 on Hemodynamics in Survival in a Rabbit Model of Hemorrhagic Shock and Retransfusion.

12. PERSONAL AUTHOR(S)
Mayer, David C., and Artman, Michael

13a TYPE OF REPORT	13b TIME COVERED	14 DATE OF REPORT (Year, Month, Day)	15 PAGE COUNT
Annual	FROM 7/89 TO 6/90	1990, July 1	18

16 SUPPLEMENTARY NOTATION

17 COSATI CODES		
FIELD	GROUP	SUB-GROUP
08		

18 SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
Hemorrhagic Shock; Reperfusion Injury; Poloxamer 188

19 ABSTRACT (Continue on reverse if necessary and identify by block number)

The effects of an antithrombotic, rheologic agent (Poloxamer 188) were examined in an acute model of severe hemorrhagic shock in rabbits. Anesthetized rabbits were instrumented for comprehensive hemodynamic monitoring. Blood was withdrawn to reduce the mean arterial pressure to 35 mmHg. The shock state was maintained for 60 minutes, followed by transfusion with autologous warmed shed blood. At the time of transfusion, treated animals received an intravenous bolus of Poloxamer 188 (200 mg), followed by continued infusion of either 50 mg/kg/hr or 200 mg/kg/hr. No demonstrable effects on hemodynamics were observed. However, in animals treated with Poloxamer 188, 13 of 16 animals survived the entire 3 hour monitoring. In contrast, in the group of animals that received the autologous blood but were not treated with Poloxamer 188, only 6 of 16 animals survived. Although additional studies are needed, these results suggest that Poloxamer 188 may favorably affect early mortality when administered in the resuscitation phase of hemorrhagic shock.

20 DISTRIBUTION/AVAILABILITY OF ABSTRACT
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21 ABSTRACT SECURITY CLASSIFICATION: (U)

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DD Form 1473, JUN 86 Previous editions are obsolete SECURITY CLASSIFICATION OF THIS PAGE

S/N 0102-LF-014-6603

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EFFECTS OF POLOXAMER 188 ON HEMODYNAMICS AND SURVIVAL IN A RABBIT MODEL OF HEMORRHAGIC SHOCK AND RETRANFUSION

ANNUAL REPORT: JULY 1, 1989 TO JUNE 30, 1990

Prepared for the Office of Naval Research
ONR Contract N00014-88-K-0429; R&T Code 441q801

Prepared by

David C. Mayer, M.D.
Michael Artman, M.D.

Department of Pediatrics
University of South Alabama
College of Medicine
2451 Fillingim Street
Mobile, Alabama 36617

INTRODUCTION

Hemorrhagic shock can result in a state in which systemic circulation and nutrient transport are insufficient to maintain vital cellular and organ functions. Besides the decrease in the volume of circulation that occurs with acute blood loss, these secondary functional alterations at the cellular and organ levels contribute significantly to the decline in microcirculatory flow.[1,2] During the shock state, the vasculature and circulating blood elements serve both as primary targets for injury and as factors that perpetuate tissue damage. The combination of low capillary flow and vascular endothelial injury resulting in exposure of collagen leads to a hypercoaguable state from increased platelet adhesiveness, release of catecholamines, acidosis, and many other factors. Disseminated intravascular coagulation may ensue via the intrinsic or extrinsic pathways, resulting in further injury to the vascular endothelium and promotion of microvascular thrombosis.[3] Furthermore, viscosity of the circulating blood is increased by all of these factors (low flow, microvascular thrombosis, and release of intracellular proteins from tissue injury) and elevated blood viscosity may directly reduce microvascular flow.[2]

Poloxamer 188 is a nonionic synthetic surfactant with the fundamental biologic activity of blocking pathologic hydrophobic interactions. The primary therapeutic potential of poloxamer 188 lies in its ability to reduce the viscosity of whole blood at concentrations that do not cause significant hemodilution,[4] which

would be of particular benefit in the treatment of hemorrnagic shock. The viscosity lowering effect of poloxamer 188 is thought to be greatest in the presence of pathologic elevations in viscosity. Poloxamer 188 has also been reported to have antithrombotic effects and to reduce platelet adhesiveness.[5] Although there is much to be learned about the mechanism of action of synthetic surfactants, they are thought to act primarily by reducing pathologic cell to cell, and molecule to molecule adherent interactions. Based upon the recognized rheologic and microcirculatory effects of poloxamer 188, we hypothesized that it may improve survival and hemodynamics in an acute whole animal model of hemorrhagic shock and retransfusion.

METHODS

Mature male New Zealand white rabbits were anesthetized with 50 mg/kg i.v. of pentobarbital sodium and then supplemented as necessary throughout the experiment. A tracheostomy was performed and mechanical ventilation instituted with room air. The animals were kept on a warming blanket and under heating lamps to maintain a physiologic rectal temperature (recorded continuously). Continuous ECG monitoring was done throughout the experiment. A femoral cutdown was performed for the introduction of catheters into the descending aorta and inferior vena cava via the femoral artery and vein. The chest was opened in the midline so that catheters could be placed directly into the right atrium, left

ventricle, and main pulmonary artery. An ultrasonic flow probe was fitted onto the ascending aorta. These surgical procedures required no more than 35 minutes to complete. This preparation allowed for continuous monitoring of heart rate and pressures in the descending aorta, right atrium, main pulmonary artery, and left ventricle. Ascending aortic flow was monitored continuously and was used to determine stroke volume and cardiac output. The maximum rate of left ventricular pressure development (LV dP/dt) was derived from the left ventricular pressure curve. Systemic vascular resistance was calculated from the pressure and flow data. Arterial blood gas measurements were determined every 30 minutes.

Following surgery, the animals were allowed to stabilize for 30 minutes. Control hemodynamic parameters and blood gases were recorded at the end of this 30 minute period. The animals had blood withdrawn (into a warmed reservoir that contained 100 U/kg of heparin) to reduce the mean arterial pressure to 35 mmHg initially. Additional blood was not withdrawn during the following 60 minute shock period and the intrinsic physiologic compensatory mechanisms were allowed to compensate for this acute loss. Following the 60 minute shock period, the animals were then randomly assigned to one of five experimental groups (Table 1). All animals in the experimental groups were monitored a total of 3 hours (60 minutes of shock + 120 minute intervention period). The SHOCK group was not retransfused. The animals in the other 4 experimental groups were given a warmed autologous transfusion (over 10 minutes) beginning 60 minutes after the initiation of shock. The animals in

the TRANS group were retransfused only. The animals in the VOL group were retransfused with a volume of normal saline equivalent to the volume of drug given in the next 2 experimental groups (10 ml/kg bolus and 5 ml/kg/hr maintenance). The animals in the LOW and HIGH drug groups were given an i.v. bolus of 200 mg/kg of poloxamer 188 over 5 minutes 60 minutes after the initiation of shock. At the time of retransfusion, animals in the LOW drug group were begun on a maintenance i.v. infusion of poloxamer 188 at a rate of 50 mg/kg/hr; animals in the HIGH drug group received poloxamer 188 at a rate of 200 mg/kg/hr. Animals in the CONTROL group were instrumented and monitored for 3 hours, but were not bled or transfused. Hemodynamic parameters and blood gases were recorded in all groups every 30 minutes throughout the 3 hour monitoring period. Animals that survived to the end of the 3 hour monitoring period were sacrificed by an i.v. overdose of pentobarbital.

Comparisons among groups for the hemodynamic and pH data were made by analysis of variance (ANOVA) with Fisher's LSD test (or Newman-Keuls for markedly disparate sample sizes) when the f value from the analysis of variance indicated a significant difference was present. Comparisons between groups for the survival data were made using Fisher's Exact test. Statistical significance was defined as $p<0.05$.

RESULTS

Figure 1 presents the data on the mean aortic pressure for all 6 groups. The animals in the surgery CONTROL group (C) had no significant change in mean aortic pressure throughout the monitoring period, and all of the animals in this group survived the 3 hour monitoring period. The animals in all 5 experimental groups had a marked reduction in pressure 5 minutes after the initiation of shock, but without further intervention intrinsic compensatory mechanisms allowed for recovery to 60-70 mmHg by 30 minutes after blood withdrawal. The animals in the groups that were retransfused at 60 minutes-RETRANS (R), VOL (V), LOW (L), and HIGH (H)-demonstrated no significant change or intergroup variation in mean aortic pressure throughout the remainder of monitoring period. The animals in the SHOCK (S) group were not retransfused and showed a significant decline in mean aortic pressure from 60 to 150 minutes after the initiation of shock; none of the group S animals survived the full 180 minute monitoring period.

Figures 2-5 present the arterial pH data. Figure 2 shows the results for the S, R, V, and C groups. The animals in the surgery control group (C) demonstrated no significant reduction in arterial pH during the 3 hour period of instrumentation and anesthesia. For the first 90 minutes of the monitoring period there was a gradual but significant decline in arterial pH in the 3 experimental groups shown on this figure (groups S, R, and V). 90 minutes after the

initiation of shock, all of the animals in the 5 experimental groups were still alive (n=8 for each group). Following 90 minutes, there was a decrease in survival in all the experimental groups, particularly in the S, R, and V groups. As figure 2 demonstrates, the animals in the R and V groups that survived 120 minutes after the initiation of shock had a significantly higher arterial pH compared to the animals in group S. In order to evaluate this finding, we compared the arterial pH of 3 hour survivors versus non-survivors in all 5 experimental groups at 60 minutes into shock (Fig 3). At this time, the animals in all 5 experimental groups were theoretically equivalent because there had been no interventions. However, figure 3 demonstrates there was a significant difference in the arterial pH between survivors and non-survivors at this time. Figure 4 subdivides the 3 hour survivors into those that received the drug and those that did not and again compares these groups to the 3 hour nonsurvivors 60 minutes into shock and before any interventions. Figure 4 demonstrates an even greater difference in arterial pH between the 3 hour TRANS & VOL survivors and the 3 hour non-survivors at 60 minutes into shock. Figure 3 also shows a difference between the arterial pH of the non-survivors and the survivors that received the drug, however this difference was not found to be statistically significant. Figure 5 presents the data on mean arterial pH for the SHOCK (S), LOW (L), and HIGH (H) groups. There was a gradual and significant decline in mean arterial pH in all 3 groups throughout the 3 hour monitoring period (compared to surgery

control-not shown on fig. 5). Although there appears to be an attenuation in the decline in mean arterial pH in the HIGH dose drug group from 90 to 180 minutes this was not significantly different from the other interventional groups (R, V, and L groups).

Figure 6 illustrates the results obtained for cardiac output in the 5 experimental groups (expressed as percent of baseline measured at time 0). All 5 groups demonstrated a marked decrease in cardiac output to 30-40 % of baseline 5 minutes into the shock period. There was a modest recovery to 45-60 % of baseline during the remainder of the shock period as measured at 30 and 60 minutes. Although figure 6 demonstrates a significant improvement in the cardiac output in the HIGH (H) dose drug group 30 minutes after retransfusion and administration of the drug (time 90) compared to the SHOCK (S) group, this improvement was not significantly different from the other intervention groups (groups R, V, and L) and this improvement was not sustained throughout the remainder of the monitoring period (120 to 180 minutes).

Table 2 presents the data on survival of the 3 hour monitoring period for all 5 experimental groups. Although the survival in the LOW (L) and HIGH (H) drug groups was significantly improved compared to the SHOCK (S) group, there was no significant difference in 3 hour survival between any of the intervention groups (TRANS, VOL, LOW, and HIGH).

In summary, poloxamer 188 did not significantly affect arterial pH at any time following retransfusion in this model. With the

possible exception of cardiac output 30 minutes after retransfusion and administration of the drug in the high dose drug group (group H), poloxamer 188 did not significantly affect hemodynamic variables following transfusion for hemorrhagic shock. Finally, the addition of poloxamer 188 did not significantly improve 3 hour survival compared to the groups (RETRANS and VOL) that received standard forms of therapy in this model of hemorrhagic shock and retransfusion.

DISCUSSION

Previous studies have reported that poloxamer 188 has antithrombotic properties, rheologic activity, and beneficial effects on microcirculatory flow. The antithrombotic effects were demonstrated in a porcine model involving the placement of coronary artery wire coil stents by balloon angioplasty. Animals were sacrificed 4 hours after the procedure and autopsy findings demonstrated a significant reduction in thrombus formation in the poloxamer 188 treated animals compared to controls.[5] Similar antithrombotic action was reported utilizing a rabbit model of amniotic fluid embolism.[6] Poloxamer 188 reduces the viscosity of whole blood at low shear rates without causing significant hemodilution.[7,8] This effect is found to be greatest in situations of pathologic elevations in viscosity caused by circulating fibrinogen-fibrin complexes.[9] The proposed mechanism for this

observation is that poloxamer 188 binds to hydrophobic portions of the fibrin molecule which prevents the development adhesive frictional forces in the microcirculation. Poloxamer 188 has also demonstrated beneficial effects on microvascular blood flow in a whole rabbit model of stroke,[10] utilizing middle cerebral artery occlusion followed by reperfusion.

The effects of poloxamer 188 on circulatory dynamics has been previously reported by Grover et al..[11] This study used mongrel dogs that were extensively instrumented for hemodynamic monitoring. The animals were bled to a mean aortic pressure of 50 mmHg and then were immediately transfused with 1ml/kg of a 5% poloxamer 188 solution (exp) or normal saline (control). This study reports no significant differences between control and poloxamer 188 groups in cardiac output, mean arterial pressure, total peripheral and pulmonary resistances, left ventricular work, or arterial pH. Only renal artery flow was significantly improved 30 minutes after infusion of poloxamer 188 in the experimental group. Survival was not addressed in this report. Our study did not demonstrate any significant differences in hemodynamic parameters, arterial pH, or survival to 180 minutes after the initiation of shock between the drug treated groups and the groups that received the traditional therapies for hemorrhagic shock (whole blood transfusion with or without the addition of extra volume of normal saline).

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ANIMAL GROUPS

All groups (except C) shocked 60 min. Monitored 3hr.

Group		Description
Experimental		
SHOCK	(n=8)	Shock only.
RETRANS	(n=8)	Autologous transfusion.
VOL	(n=8)	Autologous transfusion with volume of saline equivalent to volume of drug given.
LOW	(n=8)	IV bolus (200mg/kg) followed by cont. infusion poloxamer 188 (50mg/kg/hr) with transfusion.
HIGH	(n=8)	IV bolus (200mg/kg) followed by cont. infusion poloxamer 188 (200mg/kg/hr) with transfusion.
Surgery Control		
CONTROL	(n=6)	Surgery only.

TABLE 1 ANIMAL GROUPS

MEAN AORTIC PRESSURE

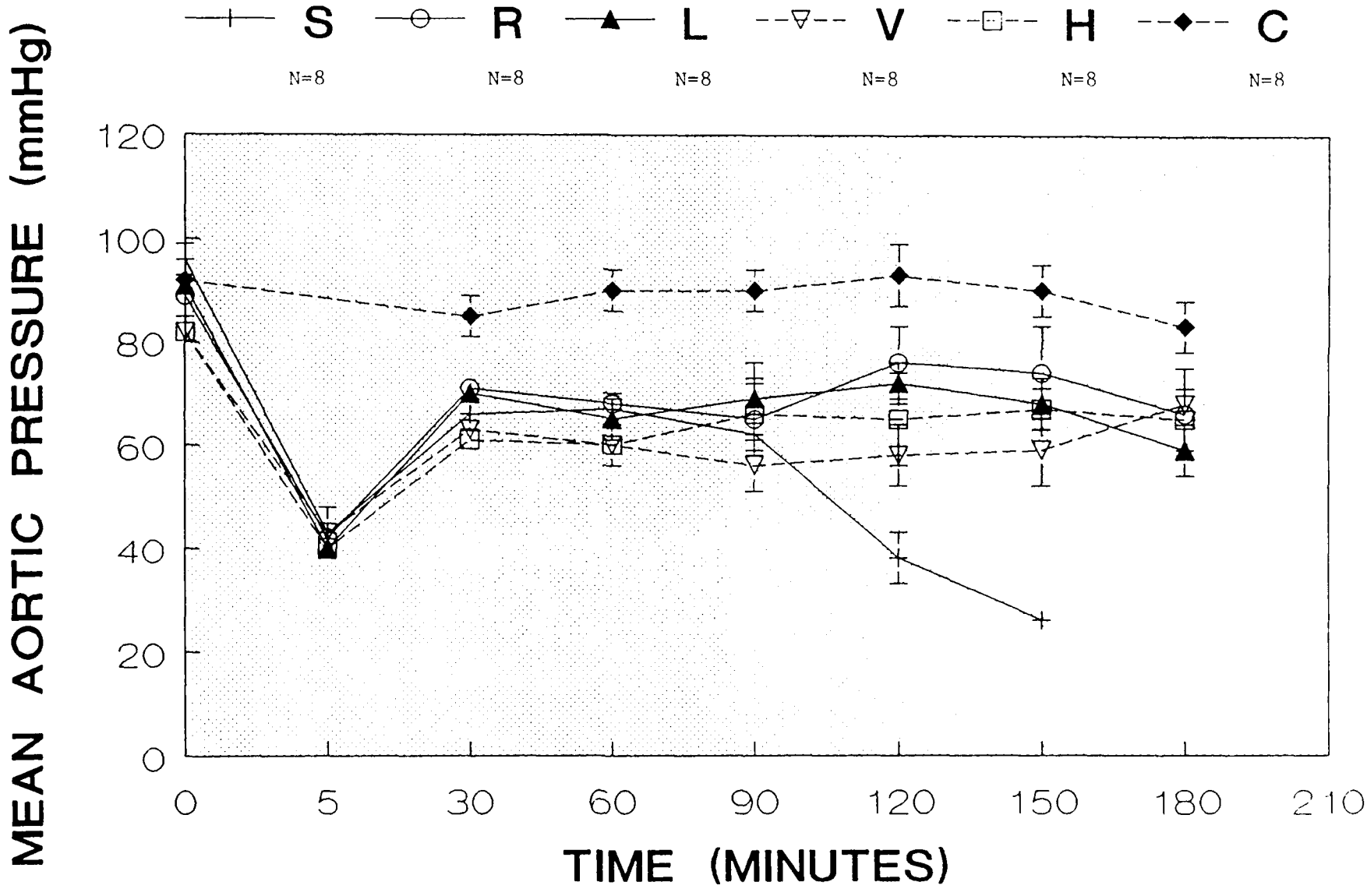

FIGURE 1 MEAN AORTIC PRESSURE

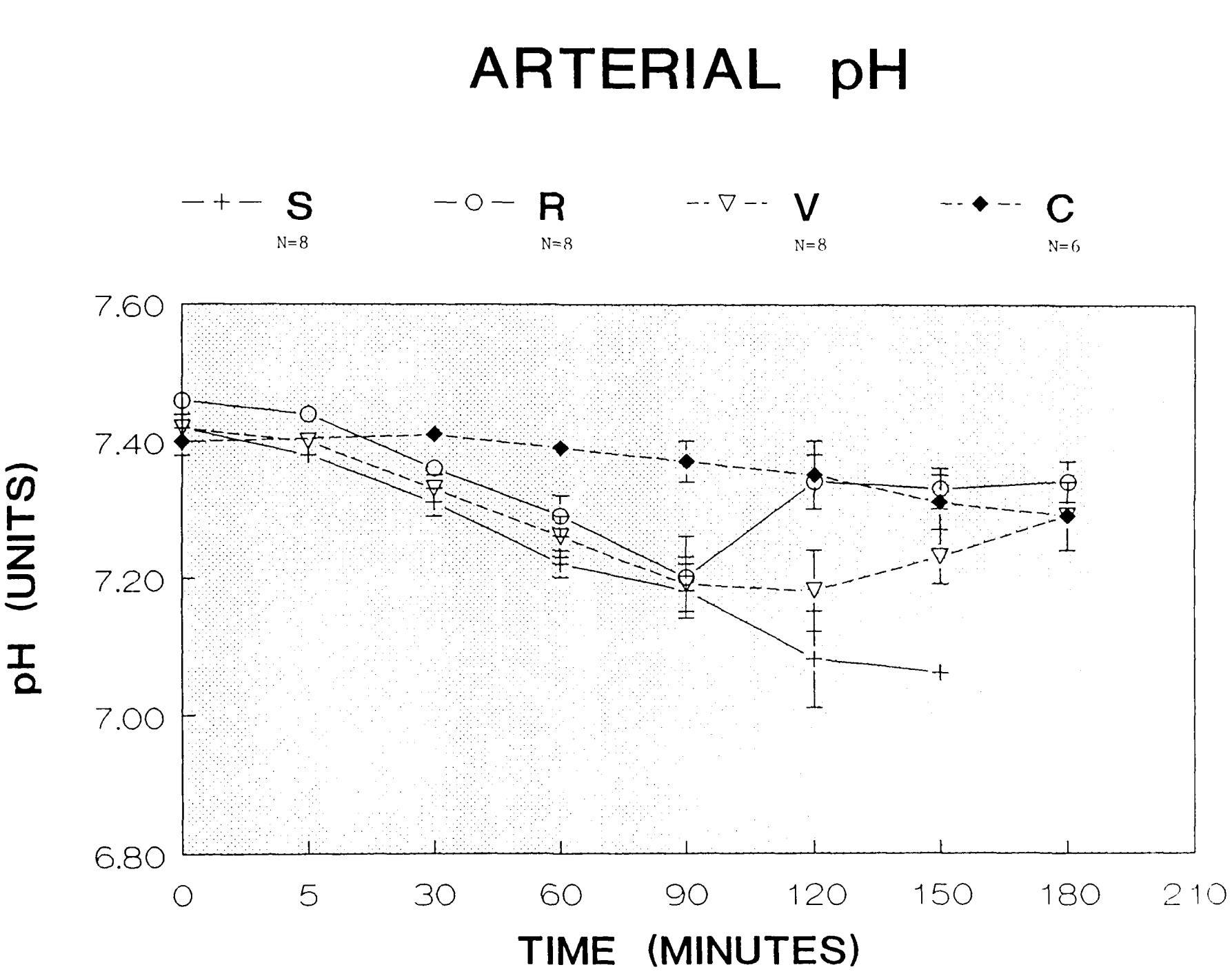

FIGURE 2 ARTERIAL pH: SHOCK, RETRANS, VOL, CONTROL GROUPS

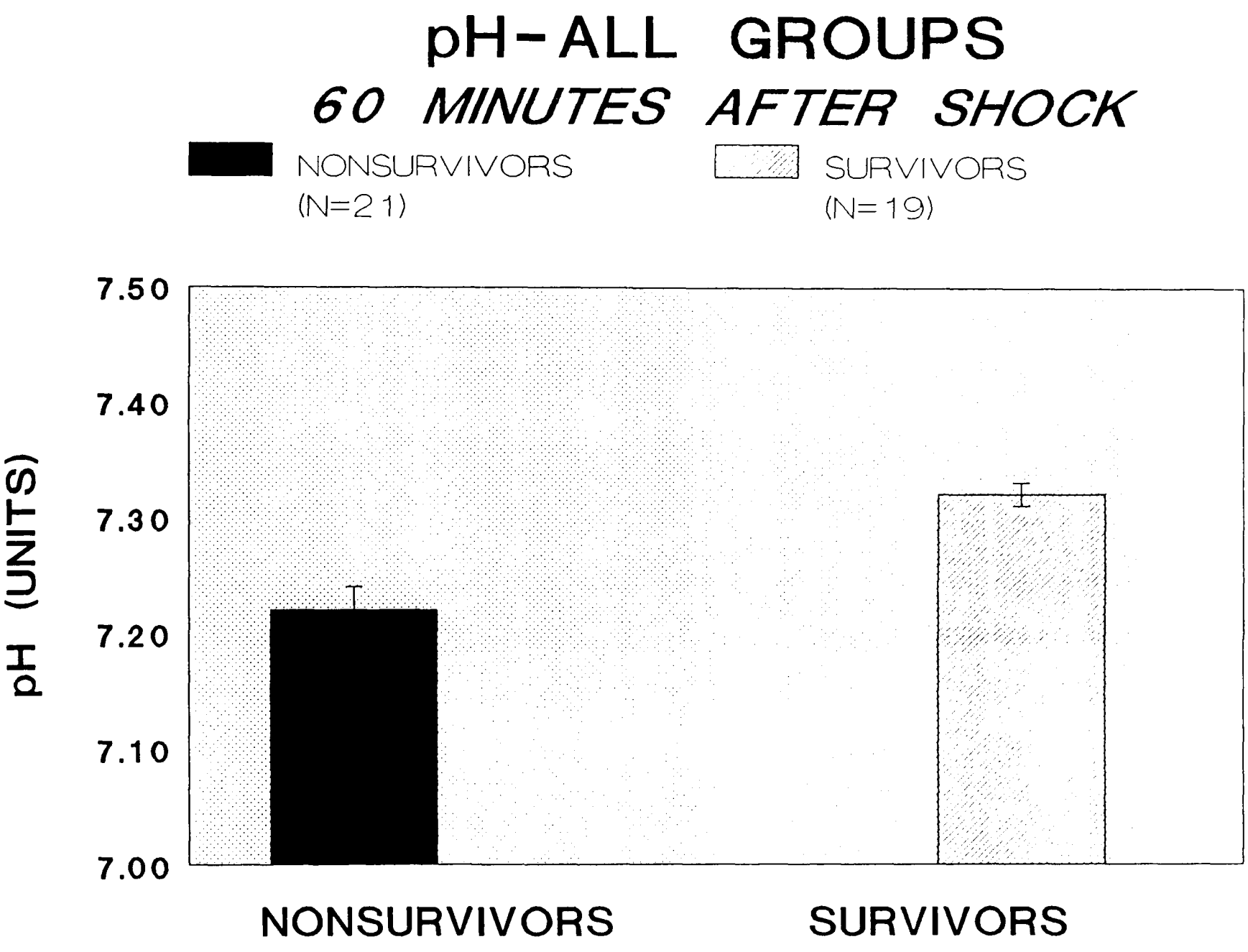

FIGURE 3 ARTERIAL pH: ALL GROUPS-SURVIVORS VERSUS NONSURVIVORS

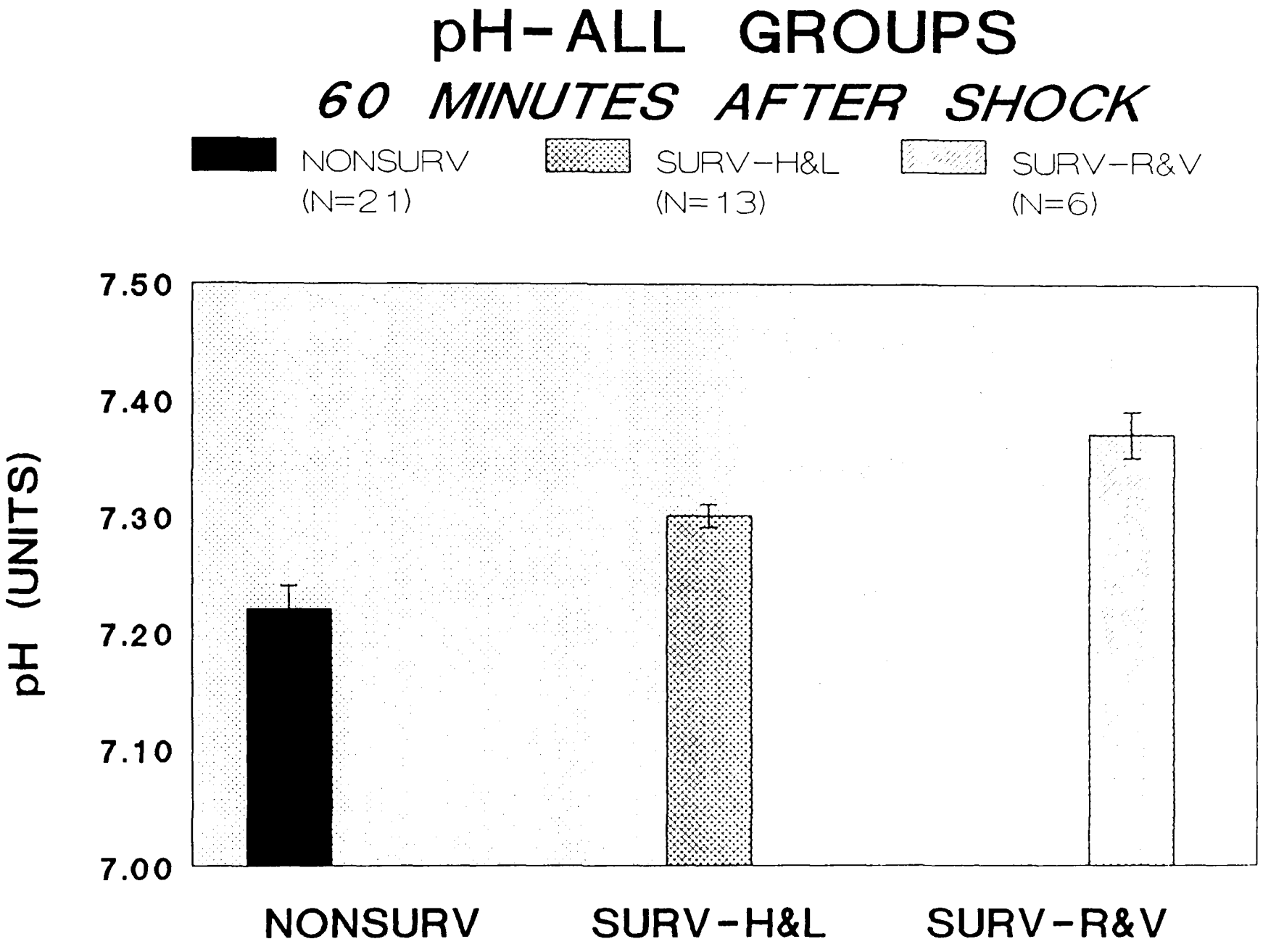

FIGURE 4 ARTERIAL pH: ALL GROUPS-SURVIVORS (R & V), SURVIVORS (H & L), AND NONSURVIVORS

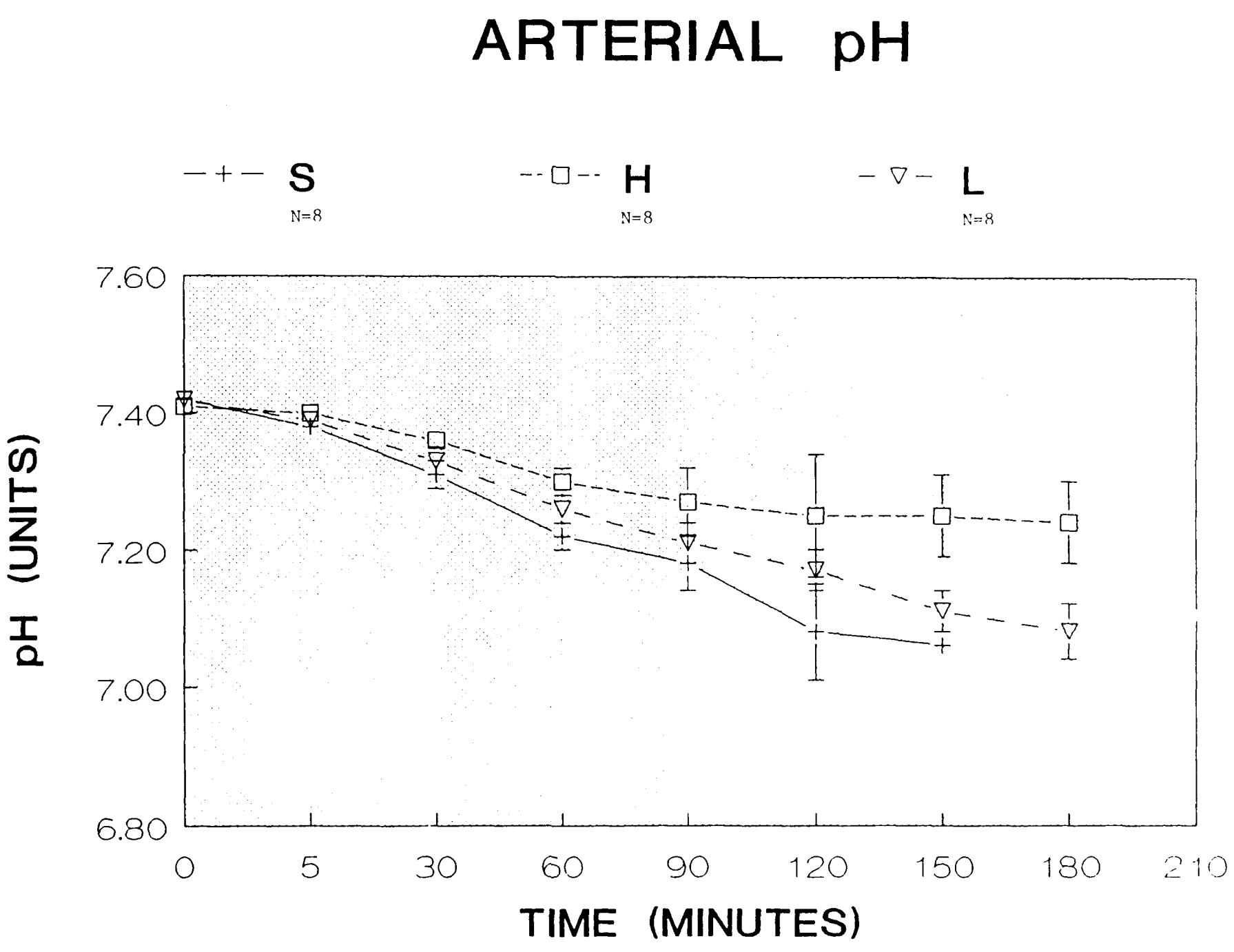

FIGURE 5 ARTERIAL pH: GROUPS SHOCK, HIGH, AND LOW

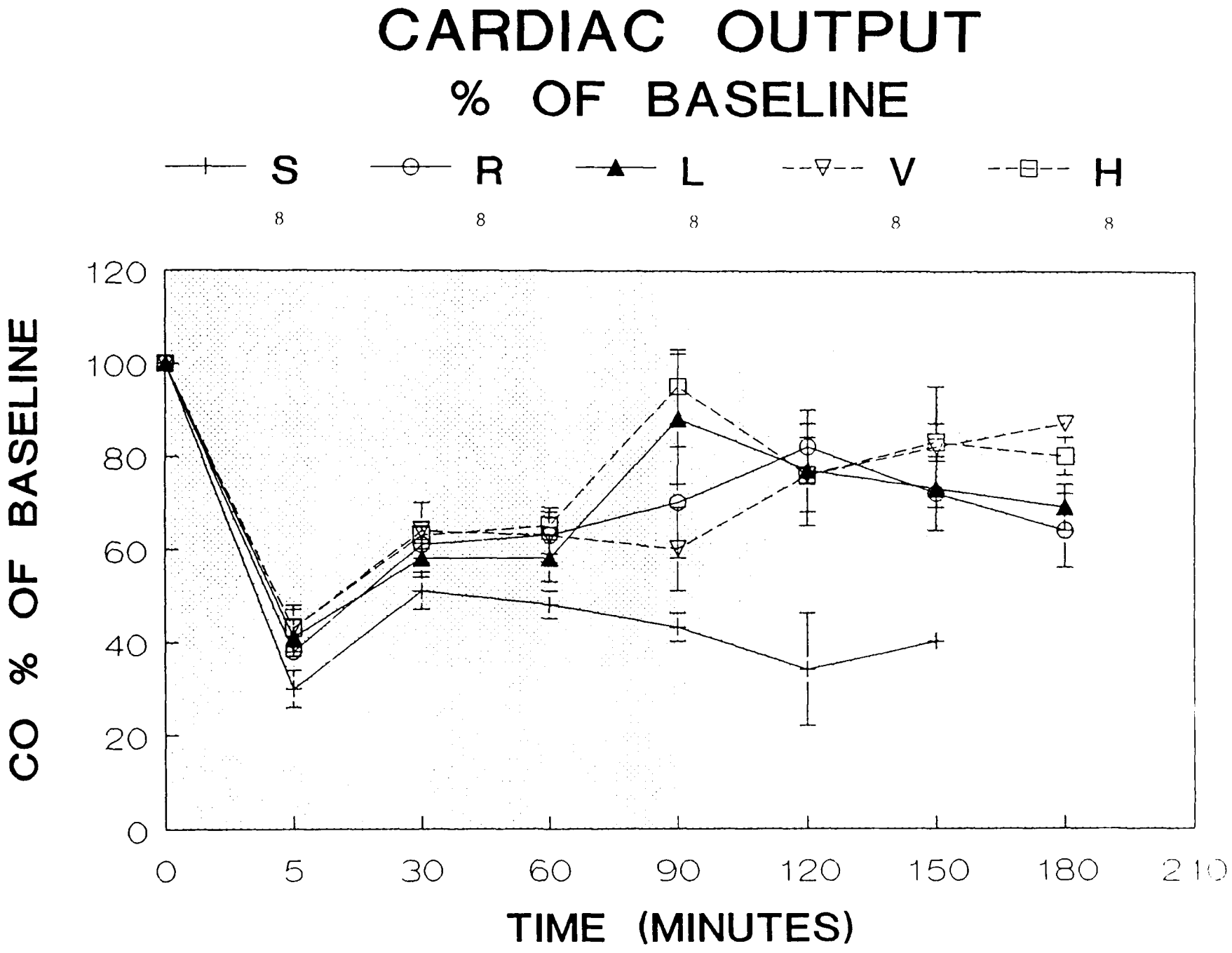

FIGURE 6 CARDIAC OUTPUT = % OF BASELINE

SURVIVAL

3 hours after initiation of shock

Group	Results
S	0/8
R	4/8
V	2/8
L	6/8*
H	7/8*

*=significantly different from S $p<0.05$

TABLE 2 SURVIVAL: 5 EXPERIMENTAL GROUPS

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